Studies on Genetic and Hormonal Sex Control in Domesticated Rainbow Trout. I. The Effect of Heat Shock Treatment for Induction of Triploidy in Cultured Rainbow Trout *(Salmo gairdneri* Richardson*)*

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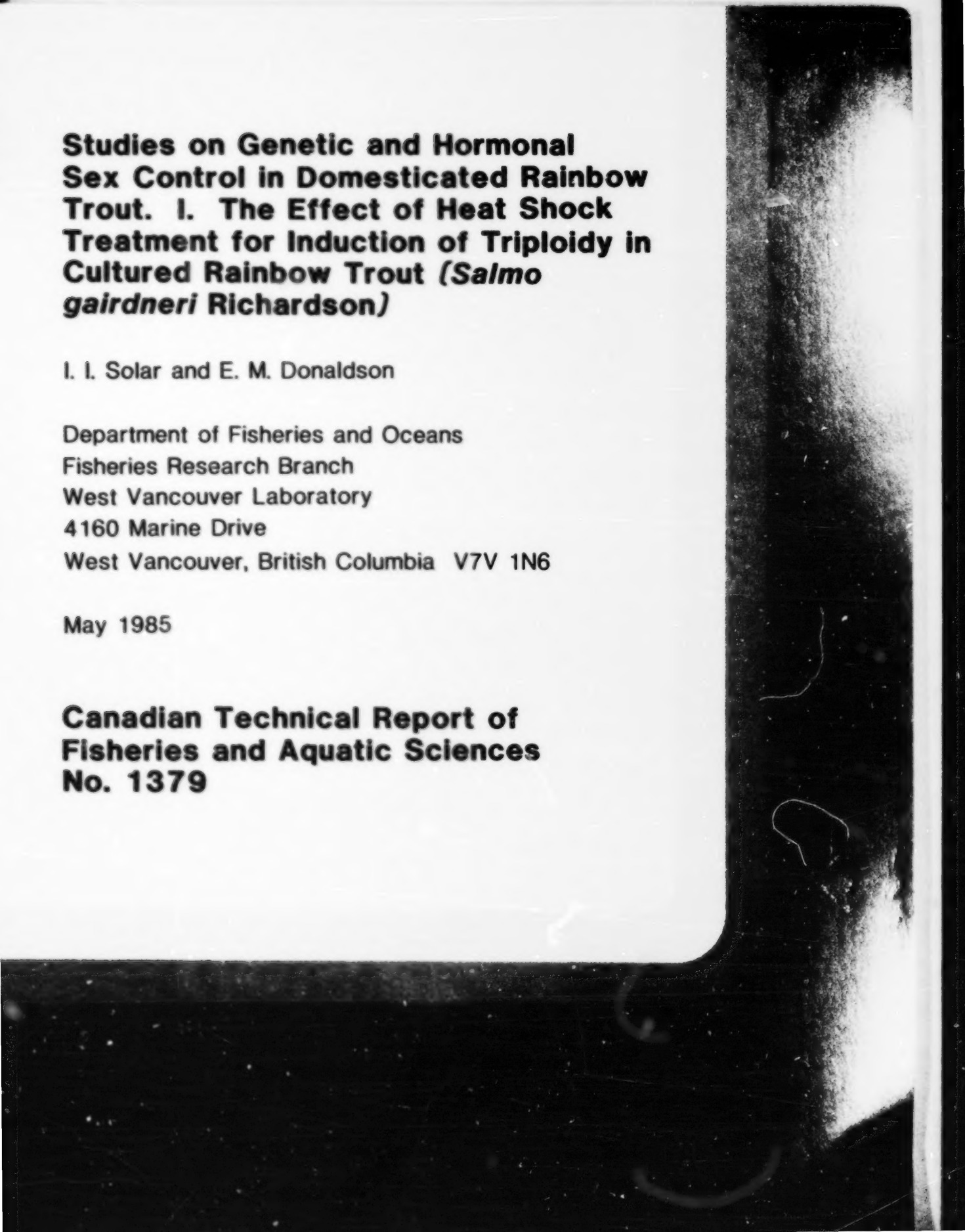

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STUDIES ON GENETIC AND HORMONAL SEX CONTROL IN DOMESTICATED RAINBOW TROUT. I. THE EFFECT OF HEAT SHOCK TREATMENT FOR INDUCTION OF TRIPLOIDY IN CULTURED RAINBOW TROUT (*Salmo gairdneri* Richardson)

by

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ABSTRACT

Solar, I. I. and E. M. Donaldson. 1985. Studies on genetic and hormonal sex control in domesticated rainbow trout. I. The effect of heat shock treatment for induction of triploidy in cultured rainbow trout (Salmo gairdneri Richardson). Can. Tech. Rep. Fish. Aquat. Sci. No. 1379: 15 p.

The effect of heat shock treatment for triploid induction and the growth performance of triploids and diploids were compared between two populations of rainbow trout, cultured (Spring Valley) and wild (Pennask Lake). Marked differences were observed in the response of the eggs regarding treatment efficiency and relative survival. Heat shock of 30°C was necessary to produce 100% triploids in the domestic stock compared with 28°C in the wild population. Survival was generally higher in the domestic trout. They had substantially better growth compared with the wild diploids and triploids, and showed no significant differences within groups in mean weight and condition factor after a 105 day period of intensive rearing which included three weeks of gradual transfer to seawater.

Key words: triploidy, heat shock, chromosomes, survival, growth, sterility, flow cytometry

RESUME

Solar, I. I. and E. M. Donaldson. 1985. Studies on genetic and hormonal sex control in domesticated rainbow trout. I. The effect of heat shock treatment for induction of triploidy in cultured rainbow trout (Salmo gairdneri Richardson). Can. Tech. Rep. Fish. Aquat. Sci. No. 1379: 15 p.

L'effet du traitement par chocs thermiques pour provoquer l'apparition de triploides et les taux de croissance des triploides et des diploides on été comparés entre deux populations de truites arc-en-ciel, l'une d'élevage (Spring Valley), et l'autre sauvage (lac Pennask). Des différences marquées ont été observées dans la réponse des oeufs en ce que concerne l'efficacité du traitement et le taux de survie relatif. Il a fallu un choc thermique de 30°C pour produire 100% de triploides dans le stock d'élevage, comparativement à 28°C dans la population sauvage. La survie était généralement plus élevée chez les truites d'élevage. Leur croissance été bien meilleure que celle des truites diploides et triploides sauvages et il n'y a pas eu de différences significatives dans le poids moyen et le facteur condition à l'intérieur des groupes arpès 105 jours d'élevage intensif conprenant trois semaines de transfert graduel à l'eau de mer.

Mots-clés: triploides, choc thermique, chromosomes, survie, croissance, stérilité, cytométrie en continue

INTRODUCTION

The control of the reproductive processes of commercially important fish species may become a useful tool in aquaculture and fisheries management (Donaldson and Hunter, 1982). Among the techniques to manipulate gonadal sex or to induce reproductively sterile stocks suitable for intensive commercial cultivation the induction of polyploidy, or increase of chromosome number, is considered a promising alternative (for review see Thorgaard, 1983).

The potential of triploid salmonids for aquaculture is based on their expected sterility. The presence of the extra set of chromosomes would cause a disruption of the normal meiotic divisions of gametogenesis resulting in a reduced gonadosomatic index, particularly of females (Purdom, 1972, in plaice x flounder; Wolters et al. 1982, in channel catfish; Lincoln and Scott, 1983, in rainbow trout; Benfey and Sutterlin, 1984, in Atlantic salmon). The benefit of the sterile condition would be realized at the time of normal maturation when the inhibition of gonadal development would allow energy to be directed towards somatic growth. The losses caused by precocious maturation, flesh deterioration and premature death, could also be reduced.

Another aspect of interest in the production of triploid fish is their presumed potential for growth. Since higher number of chromosomes cause a corresponding increase in nuclear and cellular size, triploids might be expected to be larger than their diploid counterparts of the same age. This phenomenon which is normally observed in plants does not occur in animals where in most cases the ultimate size is unaffected, presumably due to a decrease in the total number of cells (Purdom, 1972). However, in culture conditions or in sea ranching, the decrease in or absence of reproductive drive (including loss of appetite, aggresiveness, extensive migration, etc.) may result in a growth advantage and prolonged survival of triploids over the intact fish stocks.

In a previous study (Solar et al. 1984) we reported the response of a population of wild rainbow trout to the induction of triploidy by heat shock. In the present report we compare those results with the response and subsequent performance of a cultured stock to similar treatment. The use of Coulter flow cytometry to assess treatment effectiveness and the comparative performance of diploid and triploid fish to controlled feeding are also described. This stock of rainbow trout (Spring Valley) is considered well suited for triploid induction and seawater aquaculture for its favourable domestic traits, including hardiness to egg manipulation, good response to feeding initiation, fast growth and easy acclimation to seawater transfer.

MATERIAL AND METHODS

Gametes obtained from three year old broodstock of Spring Valley rainbow trout reared in net pens in seawater at the Pacific Biological

Station, Nanaimo, B.C. were transported separately to the laboratory in an insulated container with crushed ice. Pooled gametes from 4 females and 4 males were used.

FERTILIZATION AND HEAT SHOCK

Groups of approximately 200 eggs were dry fertilized with equal amounts of milt (0.25 mL) water activated at 10°C (± 0.5°C) for 30 sec, and rinsed prior to heat shock treatment. Heat shocks were applied one minute after water activation by submerging two replicates simultaneously into a thermoregulated water bath (Haake 3 type C 115 V) at the temperatures indicated in Table 1. Treatment durations were 10 min and 1 min depending on the water temperature. Control groups were submitted to the same procedures as the experimental groups except for the temperature of the water bath (11°C).

INCUBATION AND REARING

After treatment the eggs were placed in separated plexiglass boxes with 1 mm plastic mesh bottoms (Goetz et al. 1979) inserted in Heath trays supplied with 10 ± 1°C well water. Dead eggs were removed periodically and number of dead embryos recorded. Average hatch out took place at day 36 (360°C) and at swim-up (630°C) the alevins were transferred to 50 L fiberglass tanks supplied with well water at 11°C.

PLOIDY ANALYSES

Tests of ploidy levels were conducted with two flow cytometry systems. Approximately 80% of the analyses were carried out at the Department of Pathology, University of Washington School of Medicine, using a UV-based flow cytometer (ICP-22) and DAPI (4'-6- diamidino- 2 phenilindole) as DNA fluorescent stain. Methods were similar to those described by Thorgaard et al. (1982), and identical to those used in the study on wild rainbow trout reported earlier (Solar et al. 1984). The remaining samples were analysed at the Cancer Control Agency, Pharmaceutical Science Department, University of British Columbia, using a Coulter EPICS-V System UV enhanced Argon-Ion laser-based flow cytometer. For this assay small samples of blood (5-10 µl) were taken from the fish (1.5 g average weight) with a heparinized capillary tube by severing the caudal peduncle. The blood was then diluted in 1 mL heparinized physiological saline. Most samples were analysed within 3-4 h but some of them were stored for 20-22 h without apparent deterioration or cell loss. After staining with 1 mL of a solution of 0.05 mg/mL propidium iodide in 0.1% sodium citrate the samples were let to stabilize at room temperature for 15-20 min prior to analysis. DNA fluorescence was measured in 30,000 erythrocytes per sample. Data was collected, stored and analysed by Coulter

MDADS computer with ADC (analog to digital converter) and graphic display terminal for single parameter histogram presentation. Ploidy levels were determined by comparing the DNA content of cells from treated fish with that of diploid control fish.

HISTOLOGY PROCEDURES

For the observation of gonadal condition and determination of sex, histological sections were cut and stained with hematoxylin-eosin.

GROWTH AND FEED CONVERSION ASSAY

At 11 months post-hatch 2 groups of triploid fish and 2 groups of controls (30 fish per group) were selected for similar average weight (approximately 43 g) and acclimated for 10 days in 200 L tanks supplied with well water (9.5-10.5°C). After 6 weeks the fish were transferred to 1100 L tanks, vibrio vaccinated and gradually transferred to full salinity seawater over a 3 week period. The fish were fed to satiation twice daily and amounts of feed (Oregon moist pellets) consumed recorded daily. Weight and fork length were measured every third week for 15 weeks.

RESULTS

TRIPLOIDY INDUCTION

Heat shock at 30°C for 10 min, 1 min after fertilization produced 100% triploids. Treatments at lower temperatures were less effective while the 24°C shock for 10 min and the 35°C for 1 min failed to induce triploidy (Table 1). The use of the ICP-22 and EPICS-V flow cytometry systems proved reliable and accurate for the measurement of erythrocyte DNA fluorescence to identify triploids. The mean channel locations for triploids and diploids in the ICP-22 were 137.3 ± 5.3 and 92.0 ± 3.8, respectively (3n/2n ratio: 1.49). The mean channel locations for triploid and diploids in the Coulter EPICS-V were 60.1 ± 1.6 and 39.5 ± 2.5 (3n/2n ratio: 1.52).

SURVIVAL AND GROWTH

Computation of dead eggs where no embryonic development was apparent by microscopic examination and the count of abortive and surviving embryos

during incubation permitted the estimation of average activation rate as 91.5%. Up to the eyed stage survival of the treated eggs was not significantly different from controls. However, from that stage on through hatch out numerous embryos failed to proceed through normal development, resulting in drastic reduction of survival, particularly in the groups treated at 28 and 30°C (Fig. 1). Egg and alevin survival in the groups treated with 24 and 35°C were lower but not significantly different from controls.

Significant differences in growth were observed between controls and the experimental groups containing variable numbers of triploid fish (22-100%) at 8 months of age. These differences however, are mostly attributable to variable load densities in the culture tanks caused by treatment related differential mortalities. Data obtained from controls and experimental groups combined based on statistical homogeneity (t-test of independent means) is presented in Table 2. Additional t-test mean comparison of measurements from fish of known ploidy (grouped after ploidy levels were determined for individual fish) demonstrate that the means of weight, length and condition factor (K), and the frequency of size (weight) distributions (Fig. 2) are similar for diploid and triploid Spring Valley rainbow trout at 8 months of age.

The results of the growth experiment conducted for 105 days under controlled feeding conditions corroborate our earlier observations of similarity between the growth performance of juvenile diploid and triploid Spring Valley rainbow trout. At the end of the experiment the mean weight, growth rate and feed conversion efficiency of the triploid fish were lower but not significantly different from controls (Table 3).

GONADAL DEVELOPMENT

Histological analysis of the gonads of 8 month old fish revealed a close to normal sex ratio, 1.3 and 1.4 to 1, male/female in diploid and triploid fish, respectively. Diploid and triploid testes had similar cytological structure, showing apparently normal developing spermatogonia. The ovaries of the triploid females were composed only of arrested oogonia, whereas the diploid ovaries were packed with oocytes at the perinucleolar stage.

DISCUSSION

The Spring Valley stock of rainbow trout (S.V.) compared favourably in relation to the wild population from Pennask Lake (P.L.) assayed previously in responsiveness to heat shock treatment for the induction of triploidy. Survival was consistently better in the S.V. fish than in the P.L. triploids at all the temperatures assayed. The curve of percentage of triploids induced showed a similar pattern in both populations with the S.V. curve shifted

approximately two degrees Celcius higher to produce a comparable percentage of triploids. The loss of groups of eggs treated at 29°C, due to failure of the water supply system during incubation, prevented us from finding whether 100% triploids could have been produced at that temperature, possibly with better results than the 18% survival obtained at 30°C. The difference in the thermal level required to induce triploids in the Spring Valley trout eggs may be attributed in part to their comparative larger average size (0.095 g S.V. vs 0.085 g P.L.). The P.L. eggs, being smaller may have permitted more efficient heat transfer (Scheerer and Thorgaard, 1983). Another factor could be that the P.L. trout were collected from a cold water lake located at 1420 m altitude in the B.C. interior, whereas the Spring Valley trout are a domestic stock adapted to warmer hatchery water conditions.

Our results are in agreement with those reported by other workers in this species (Chourrout, 1980; Lincoln and Scott 1983). However, our 34 and 35°C for 1 min heat shocks were unsuccessful for induction of triploidy in S.V. trout. This result differs from the 11-23% triploids obtained by Thorgaard et al. (1981) at the same temperatures in rainbow trout from the Spokane Trout Hatchery (Washington, U.S.A.). Intraspecific variability may account for this discrepancy. Chourrout and Quillet (1982) and Scheerer and Thorgaard (1983) have reported high survival rates in rainbow trout while inducing 100% triploids, using 10 min heat shocks at 26 and 28°C (63 and 97% respectively, not different from controls). On the other hand, the same treatment (28°C) applied by Scheerer and Thorgaard on eggs from different females yielded 0 to 60% triploidy accompanied by poor survival (16 to 53%). The inconsistency of the various reported results suggests that the treatment efficiency and the survival of the treated eggs is very much dependent on the combined effect of individual condition, the quality of the eggs, and the intensity of the treatment applied.

The differences between the two populations in triploid growth and general performance were remarkable. One year old Spring Valley trout were more than three times larger than Pennask Lake fish of the same age reared under similar conditions of temperature and loading density. Furthermore, triploid Spring Valley showed similar growth compared with the controls, whereas P.L. triploids grew at a rate about 1/3 of that of the diploid controls.

The comparable parallel growth of diploid and triploid Spring Valley trout observed in this study contrasts with previous reports (including our own on wild rainbow trout) which consistently attribute triploid rainbow trout with significantly slower growth. The current result increases the promise and potential for aquaculture of triploid fish of this species when the appropiate strain is chosen.

The relatively short duration of the growth study (105 days) and the fact that it did not cover the period of sexual maturation do not allow for definite conclusions to be drawn in this regard. Further observation of the fish performance under normal rearing conditions in seawater over the period of sexual maturation could permit additional comparisons.

The consistent inhibition of ovarian development observed in triploid female trout (Thorgaard and Gall, 1979; Lincoln and Scott, 1983; Solar et al. 1984) parallels similar reports by other workers on the females

of Atlantic salmon (Benfey and Sutterlin 1984) flatfish (Purdom 1972), and catfish (Wolters et al. 1982). A complete lack of developing oocytes is the norm in rainbow trout, whereas a few or several oocytes have usually been observed in the ovaries of triploid individuals of the other species mentioned.

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Table 1. Percentage of hatched and feeding fry (as percent of activated eggs and relative to controls). Mean ± SD. Percentage of triploidy induced.

Treatment °C	Initial number of eggs	Survival Hatch-out D 36	Feeding D 100	S	N	% Triploid
11[a](control)	240.0 ± 2.1	94.4 ± 1.4	88.3 ± 0.8	-	40	0.0
24[a]	216.5 ± 0.7	83.0 ± 10.4	79.4 ± 8.0	N.S.	40	0.0
26[a]	204.5 ± 0.7	80.0 ± 1.3	67.7 ± 2.7	**	40	22.5 ± 10.6
28[a]	197.5 ± 0.7	50.5 ± 4.7	40.9 ± 1.7	**	36	86.1 ± 2.8
30[a]	183.0 ± 8.7	24.3 ± 7.5	17.9 ± 4.6	**	24	100.0 -
34 - 35[b]	292.5 ± 10.6	86.9 ± 5.1	82.4 ± 5.6	N.S.	40	0.0

[a] Treatment duration: 10 min

[b] Treatment duration: 1 min

** $P < 0.01$

Table 2. Length, weight, condition factor, survival and load density for combined data of groups containing all diploid and variable numbers of diploid and triploid fish at 8 months of age. Comparison of data from all diploid and all triploid fish sharing the same tank (means ± SD).

	Diploids only[a]	3n/2n groups[b]	2n[c]	3n[d]
Length	8.8 ± 0.9	9.5 ± 1.1*	9.4 ± 1.1	9.4 ± 1.4
Weight	7.6 ± 2.3	9.6 ± 3.2*	9.4 ± 3.2	9.1 ± 3.7
Condition factor (K)[4]	1.06 ± 0.05	1.05 ± 0.04	1.07 ± 0.08	1.04 ± 0.09
% Survival	84.7 ± 3.8	49.3 ± 24.8	-	-
Load density g/l	40.1 ± 6.1	28.4 ± 7.0	-	-

* Significantly different $P < 0.01$

[a] Includes controls and 24°C treated groups (no triploids)

[b] Includes 26-28°C treated groups (22-86% triploids)

[c] Includes controls and diploid fish sharing tank with triploid fish

[d] Triploid fish only

[e] $K = (W/L^3) \times 100$

Table 3. Performance of diploid and triploid rainbow trout fed Oregon moist pellets for 105 days (temp = 9-11°C). Gradual acclimation to seawater during days 54 - 75.

	Diploid	Triploid
Initial mean weight g	41.3 ± 6.1	45.9 ± 12.8
Final mean weight g	160.5 ± 31.5	144.3 ± 50.2
Condition factor (K)	1.40	1.40
% wt gain per day	2.88	2.04
Feed conversion (F.C.)	1.46	1.80

$K. = W/L^3 \times 100$

F.C. = wet wt feed/wet wt gain

FIGURES

Fig. 1. Comparison of survival (feeding fry) and triploid induction in wild and domestic rainbow trout treated as eggs with 10 min heat shocks over the temperature range 24 to 30°C (11°C - control).

FIG. 1

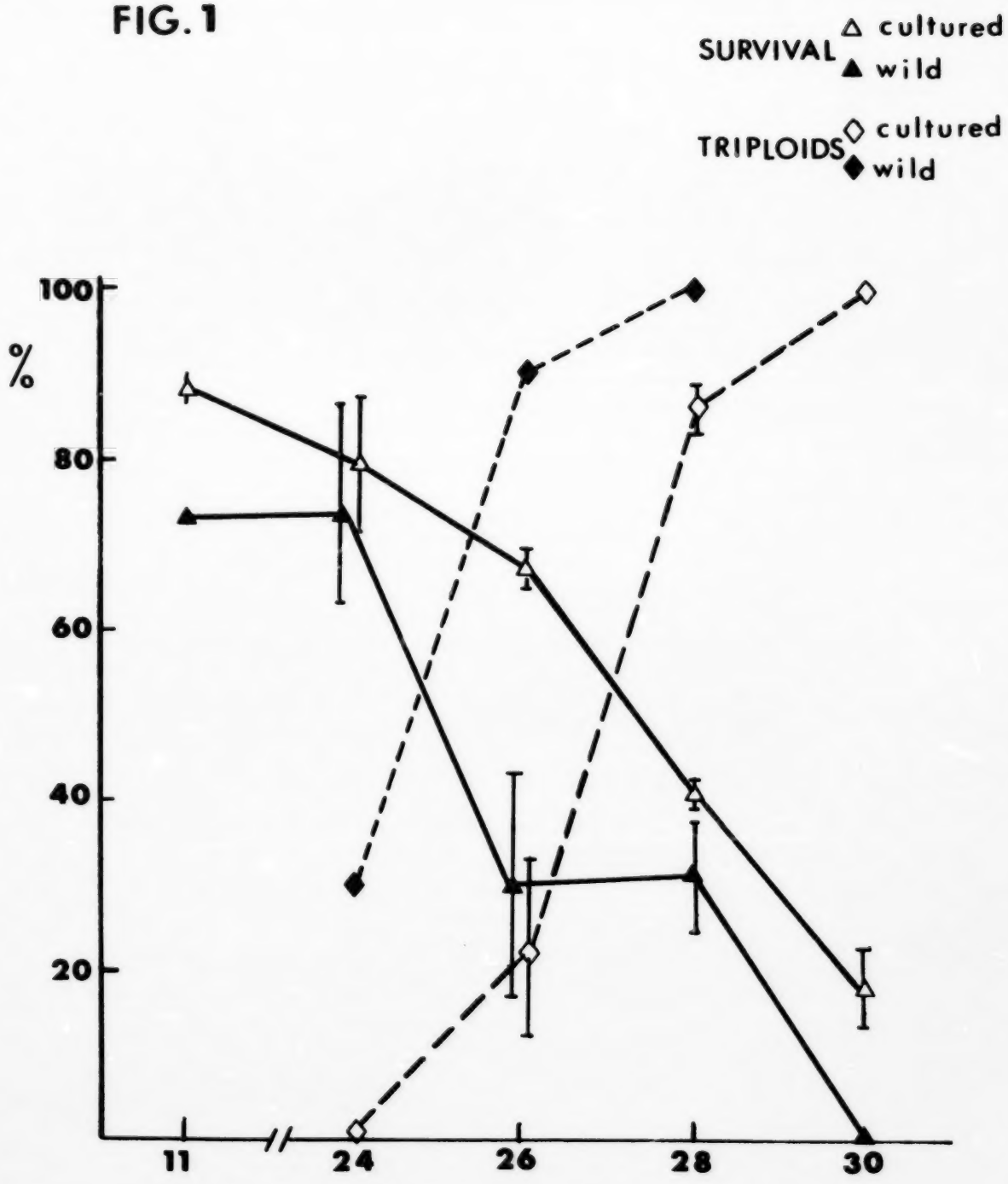
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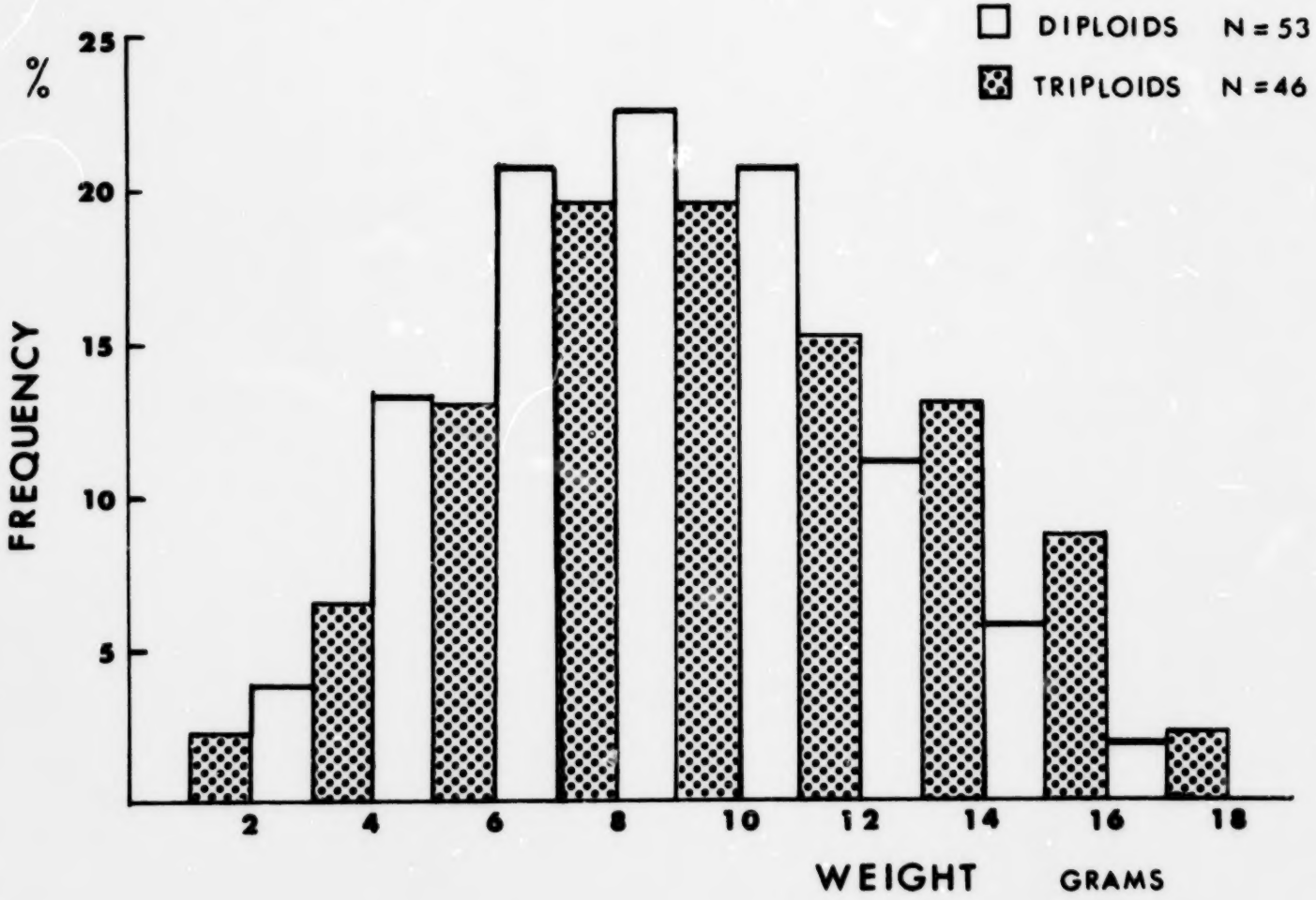
Fig. 2. Distribution of size (weight) of diploid and triploid Spring Valley rainbow trout at 8 months of age.

FIG. 2


DIPLOIDS N = 53
TRIPLOIDS N = 46
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WEIGHT GRAMS